10/728766

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=> d his

     (FILE 'HOME' ENTERED AT 15:26:25 ON 15 FEB 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:26:49 ON 15 FEB 2007
L1         28644 S THIOREDOXIN
L2        845647 S FUSION OR (PRE(W) STAINED (3W)LADDER) AND L1
L3         50640 S (TRUNCATED OR DELETION) AND L2
L4             3 S INCLUSION (W)PARTNER?
L5       8176504 S CLON? OR EXPRESS? OR RECOMBINANT
L6         39309 S L3 AND L5
L7           738 S L6 AND INCLUSION
L8             2 S (BACTERIAL (W)HOST?) AND L7
L9            59 S L7 AND (MOLECULAR (W)WEIGHT)
L10           24 DUP REM L9 (35 DUPLICATES REMOVED)
                 E CHATTERJEE D/AU
L11         1115 S E3
                 E LONGO M/AU
L12          739 S E3
                 E FLYNN E/AU
L13          291 S E3
                 E OBERFELDER R/AU
L14           56 S E3-E7
L15         2194 S L11 OR L12 OR L13 OR L14
L16            0 S L7 AND L15
L17            4 S L1 AND L15
L18            3 DUP REM L17 (1 DUPLICATE REMOVED)
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 NEWS  1          Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2          "Ask CAS" for self-help around the clock
 NEWS  3  OCT 23  The Derwent World Patents Index suite of databases on STN
                  has been enhanced and reloaded
 NEWS  4  OCT 30  CHEMLIST enhanced with new search and display field
 NEWS  5  NOV 03  JAPIO enhanced with IPC 8 features and functionality
 NEWS  6  NOV 10  CA/CAplus F-Term thesaurus enhanced
 NEWS  7  NOV 10  STN Express with Discover! free maintenance release Version
                  8.01c now available
 NEWS  8  NOV 20  CA/CAplus to MARPAT accession number crossover limit increased
                  to 50,000
 NEWS  9  DEC 01  CAS REGISTRY updated with new ambiguity codes
 NEWS 10  DEC 11  CAS REGISTRY chemical nomenclature enhanced
 NEWS 11  DEC 14  WPIDS/WPINDEX/WPIX manual codes updated
 NEWS 12  DEC 14  GBFULL and FRFULL enhanced with IPC 8 features and
                  functionality
 NEWS 13  DEC 18  CA/CAplus pre-1967 chemical substance index entries enhanced
                  with preparation role
 NEWS 14  DEC 18  CA/CAplus patent kind codes updated
 NEWS 15  DEC 18  MARPAT to CA/CAplus accession number crossover limit increased
                  to 50,000
 NEWS 16  DEC 18  MEDLINE updated in preparation for 2007 reload
 NEWS 17  DEC 27  CA/CAplus enhanced with more pre-1907 records
 NEWS 18  JAN 08  CHEMLIST enhanced with New Zealand Inventory of Chemicals
 NEWS 19  JAN 16  CA/CAplus Company Name Thesaurus enhanced and reloaded
 NEWS 20  JAN 16  IPC version 2007.01 thesaurus available on STN
 NEWS 21  JAN 16  WPIDS/WPINDEX/WPIX enhanced with IPC 8 reclassification data
 NEWS 22  JAN 22  CA/CAplus updated with revised CAS roles
 NEWS 23  JAN 22  CA/CAplus enhanced with patent applications from India
 NEWS 24  JAN 29  PHAR reloaded with new search and display fields
 NEWS 25  JAN 29  CAS Registry Number crossover limit increased to 300,000 in
                  multiple databases
 NEWS 26  FEB 13  CASREACT coverage to be extended
 NEWS 27  Feb 15  PATDPASPC enhanced with Drug Approval numbers
 NEWS 28  Feb 15  RUSSIAPAT enhanced with pre-1994 records

 NEWS EXPRESS  NOVEMBER 10 CURRENT WINDOWS VERSION IS V8.01c, CURRENT
               MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP),
               AND CURRENT DISCOVER FILE IS DATED 25 SEPTEMBER 2006.

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FILE 'HOME' ENTERED AT 15:26:25 ON 15 FEB 2007

=> d his

    (FILE 'HOME' ENTERED AT 15:26:25 ON 15 FEB 2007)

=> file medline embase biosis biotechds scisearch hcaplus ntis lifesci
COST IN U.S. DOLLARS                                 SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                      0.21       0.21

FILE 'MEDLINE' ENTERED AT 15:26:49 ON 15 FEB 2007

FILE 'EMBASE' ENTERED AT 15:26:49 ON 15 FEB 2007
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FILE 'SCISEARCH' ENTERED AT 15:26:49 ON 15 FEB 2007
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FILE 'HCAPLUS' ENTERED AT 15:26:49 ON 15 FEB 2007
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FILE 'NTIS' ENTERED AT 15:26:49 ON 15 FEB 2007
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FILE 'LIFESCI' ENTERED AT 15:26:49 ON 15 FEB 2007
COPYRIGHT (C) 2007 Cambridge Scientific Abstracts (CSA)


=> s thioredoxin
L1        28644 THIOREDOXIN

=> s fusion or (pre(w) stained (3w)ladder) and l1
L2       845647 FUSION OR (PRE(W) STAINED (3W) LADDER) AND L1

=> s (truncated or deletion) and l2
L3        50640 (TRUNCATED OR DELETION) AND L2

=> s inclusion (w)partner?
L4            3 INCLUSION (W) PARTNER?

=> s clon? or express? or recombinant
L5      8176504 CLON? OR EXPRESS? OR RECOMBINANT

=> s l3 and l5
L6        39309 L3 AND L5
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=> s l6 and inclusion
L7          738 L6 AND INCLUSION

=> s (bacterial (w)host?) and l7
L8            2 (BACTERIAL (W) HOST?) AND L7

=> d 1-2 ibib ab

L8    ANSWER 1 OF 2  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-03310  BIOTECHDS
TITLE:            Use of a fragment of an alpha-integrin in production of
                  recombinant proteins, particularly fusions
                  with G protein coupled receptors, for structure determination
      ;
                       recombinant protein production via plasmid
                     expression in host cell
AUTHOR:           MOUILLAC B; SEN T; BANERES J L
PATENT ASSIGNEE:  INSERM INST NAT SANTE and RECH MEDICALE; CNRS CENT NAT RECH
                  SCI
PATENT INFO:      FR 2856407 24 Dec 2004
APPLICATION INFO: FR 2003-7411 19 Jun 2003
PRIORITY INFO:    FR 2003-7411 19 Jun 2003; FR 2003-7411 19 Jun 2003
DOCUMENT TYPE:    Patent
LANGUAGE:         French
OTHER SOURCE:     WPI: 2005-033235 [04]
AB    DERWENT ABSTRACT:
      NOVELTY - Use of at least one fragment (I) of an alpha-integrin (II) in
      construction, and production, of a recombinant protein (III),
      is new.
           DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1)
      a recombinant protein (III) comprising at least one (I) and a
      protein of interest (IV); (2) use of at least one nucleic acid sequence
      that encodes (I) in construction of a sequence (IIIa) that encodes (III);
      (3) a nucleotide sequence (IIIa) that encodes (III); (4) a vector that
      contains (IIIa); (5) cells that contain (IIIa) or the vector of (4); and
      (6) producing (III) by introducing (IIIa) into a cell then
      expressing it.
           BIOTECHNOLOGY - Preferred Materials: (I) is the complete sequence
      for (II) or just part of it, preferably the N-terminal region, and is
      native or mutated, particularly to introduce a cleavage site for an
      endoprotease. Especially (I) includes at least the modules FG-GAP IV to
      VII and part of module III, and is particularly from the integrins
      alpha5, alphaV or alphaIIb. (I) preferably contains amino acids
      (numbering includes the signal peptide) 231-517 of alpha5; 211-495 for
      alphaV or 224-508 for alphaIIb. Most preferred are sequences of 288, 286
      and 286 amino acids, reproduced together with nucleic acid sequences that
      encode them. In (III), (I) is present upstream of (IV), which is
      particularly a membrane protein, preferably a G protein-coupled receptor
      and specifically a receptor for vasopressin and oxytocin; leukotrienes or
      cannabinoids, or a beta3 adrenergic receptor. (III) preferably includes a
      His 6 tag at the C terminus. Preferred Process: In method (6), the
      expressed (III) is cleaved with an endoprotease and (III), or
      (IV) released from it, is purified. (III) accumulates in
      bacterial hosts as inclusion bodies.
           USE - (I) is used for recombinant production of
      fusion proteins, especially recombinant membrane
      proteins such as G protein-coupled receptors, for subsequent analysis of
      their structures by X-ray crystallography or NMR.
           ADVANTAGE - When expressed as a fusion protein
      with (I), G protein-coupled receptors are obtained in large quantities,
      in a non-truncated suitable for direct analysis.
           EXAMPLE - The vector pET21a(+) was modified by insertion of a
      cassette containing an NdeI site; cDNA for integrin alpha5; BamHI site;
```

cDNA encoding the human vasopressin V2 receptor; EcoRI site and sequence encoding a 6His tag. The recombinant vector was introduced into Escherichia coli Rosetta and expressed after induction with isopropyl beta-D-thiogalactopyranoside. Periodically samples of cells were taken, lysed and analyzed by gel electrophoresis to indicate formation of a 65 kD fusion protein of alpha5 and V2. One ml of culture produced about 3 microgram of the receptor. (34 pages)

L8 ANSWER 2 OF 2 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1990:586094 HCAPLUS
DOCUMENT NUMBER: 113:186094
TITLE: Cysteine-free and deletion analogs of interleukin 6 for immuno-therapeutic use
INVENTOR(S): Fowlkes, Dana M.; Tackney, Charles T.
PATENT ASSIGNEE(S): University of North Carolina, USA; Imclone Systems, Inc.
SOURCE: PCT Int. Appl., 108 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9006370	A1	19900614	WO 1989-US5421		19891130
W: AU, DK, ES, FI, HU, JP, KR, SU					
RW: AT, BE, CH, DE, ES, FR, GB, IT, LU, NL, SE					
CA 2004261	A1	19900601	CA 1989-2004261		19891130
AU 9048014	A	19900626	AU 1990-48014		19891130
AU 639428	B2	19930729			
EP 446283	A1	19910918	EP 1990-900700		19891130
R: DE, ES, FR, GB, IT, SE					
JP 04503301	T	19920618	JP 1990-501355		19891130
ZA 8909203	A	19900926	ZA 1989-9203		19891201
PRIORITY APPLN. INFO.:			US 1988-278690	A	19881201
			US 1989-440624	A	19891122
			WO 1989-US5421	A	19891130

AB An interleukin 6 (IL-6) analog with all cysteines replaced by serines but retaining biol. activity and therefore useful as a therapeutic is described. The protein is manufactured by expression in Escherichia coli of a chimeric gene for a fusion protein of the analog and a carrier moiety connected by a cleavage sequence for a protease (e.g. collagenase, enterokinase, factor Xa). The lack of cysteines in the IL-6 analog prevents it from forming inclusion bodies in the bacterial host. A synthetic gene for a cysteine-free IL-6 analog was prepared by standard methods and fused to a β-galactosidase gene through a sequence encoding a collagenase-sensitive peptide. The protein was recovered from the host cells by standard methods after liberation of the IL-6 analog with collagenase. The purified protein stimulated B-cell differentiation with maximum activity at 43 pM and was also active in stimulation of bone marrow cells (delta assay). IL-6 deletion analogs with altered biol. activities are also disclosed.

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:26:49 ON 15 FEB 2007
L1 28644 S THIOREDOXIN
L2 845647 S FUSION OR (PRE(W) STAINED (3W)LADDER) AND L1
L3 50640 S (TRUNCATED OR DELETION) AND L2.

```
L4            3 S INCLUSION (W)PARTNER?
L5      8176504 S CLON? OR EXPRESS? OR RECOMBINANT
L6        39309 S L3 AND L5
L7          738 S L6 AND INCLUSION
L8            2 S (BACTERIAL (W)HOST?) AND L7

=> s l4 1-3 ibib ab
MISSING OPERATOR L4 1-3
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> d l4 1-3 ibib ab

L4   ANSWER 1 OF 3  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
ACCESSION NUMBER:   2004:219988  BIOSIS
DOCUMENT NUMBER:    PREV200400223055
TITLE:              Methods for production of proteins.
AUTHOR(S):          Chatterjee, Deb [Inventor, Reprint Author]; Longo, Mary
                    [Inventor]; Flynn, Elizabeth [Inventor]; Oberfelder, Robert
                    [Inventor]
CORPORATE SOURCE:   North Potomac, MD, USA
                    ASSIGNEE: Invitrogen Corporation, Frankfurt am Main, DE,
                    USA
PATENT INFORMATION: US 6703484 20040309
SOURCE:             Official Gazette of the United States Patent and Trademark
                    Office Patents, (Mar 9 2004) Vol. 1280, No. 2.
                    http://www.uspto.gov/web/menu/patdata.html. e-file.
                    ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE:      Patent
LANGUAGE:           English
ENTRY DATE:         Entered STN: 21 Apr 2004
                    Last Updated on STN: 21 Apr 2004
AB   The current invention provides methods for producing a polypeptide as
     inclusion bodies in bacterial host cells. The present methods are carried
     out by forming a gene construct comprising the genetic sequence encoding a
     polypeptide operatively linked to that of an inclusion
     partner protein, such as E. coli thioredoxin or a modified E. coli
     thioredoxin, such that host cells comprising the gene construct produce
     the polypeptide as intracellular inclusion bodies. The methods of the
     present invention facilitate the rapid isolation and purification of
     recombinant proteins. In addition, the present methods may be useful for
     producing polypeptides or proteins which are small and are typically
     difficult to express, as well as those proteins that are toxic to host
     cells such as E. coli. The present invention also provides plasmids,
     vectors and host cells to be used in the present invention for production
     of polypeptides, and methods of production of polypeptides using these
     vectors and host cells. The invention further provides methods for
     producing protein molecular weight ladders for use in protein gel
     electrophoresis, as well as proteins and protein molecular weight ladders
     produced by these methods.

L4    ANSWER 2 OF 3  BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
ACCESSION NUMBER: 1998-09126  BIOTECHDS
TITLE:            Production of polypeptides as inclusion bodies;
                     recombinant protein preparation by plasmid pTrc99A or
                     plasmid pTrxfus vector-mediated thioredoxin expression in
                     Escherichia coli inclusion body
AUTHOR:           Chatterjee D; Longo M; Flynn E; Oberfelder R
PATENT ASSIGNEE:  Life-Technol.
LOCATION:         Rockville, MD, USA.
PATENT INFO:      WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO:    US 1997-34658 8 Jan 1997
DOCUMENT TYPE:    Patent
```

LANGUAGE: English
OTHER SOURCE: WPI: 1998-399134 [34]
AB A new method for the preparation of a protein in the form of an inclusion body involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner (thioredoxin or modified thioredoxin), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as inclusion bodies. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or thioredoxin. (84pp)

L4 ANSWER 3 OF 3 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1998:493673 HCAPLUS
DOCUMENT NUMBER: 129:118770
TITLE: Methods for production of recombinant proteins as inclusion bodies in bacterial host cells
INVENTOR(S): Chatterjee, Deb; Longo, Mary; Flynn, Elizabeth; Oberfelder, Robert
PATENT ASSIGNEE(S): Life Technologies, Inc., USA
SOURCE: PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
WO 9830684	A1	19980716	WO 1998-US492		19980108
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW					
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG					
AU 9860209	A	19980803	AU 1998-60209		19980108
EP 963435	A1	19991215	EP 1998-903438		19980108
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO					
JP 2001512306	T	20010821	JP 1998-531177		19980108
US 2002065392	A1	20020530	US 1998-4068		19980108
US 6703484	B2	20040309			
US 2004204563	A1	20041014	US 2003-728766		20031208
US 2006269992	A1	20061130	US 2006-329418		20060111
US 2006286633	A1	20061221	US 2006-329401		20060111
PRIORITY APPLN. INFO.:			US 1997-34658P	P	19970108
			US 1998-4068	A1	19980108
			WO 1998-US492	W	19980108
			US 2003-728766	A1	20031208

AB The current invention provides methods for producing a polypeptide as inclusion bodies in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an inclusion

partner protein, such as Escherichia coli thioredoxin or a modified E. coli thioredoxin, such that host cells comprising the gene construct produce the polypeptide as intracellular inclusion bodies. Addnl. inclusion partner proteins include gene 32 protein of bacteriophage T4, KpnI methylase, and E. coli Dead-Box protein. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The desired protein can be released from inclusion bodies by chemical cleavage (CNBr or hydroxylamine) or enzymic cleavage (factor Xa, thrombin, enterokinase). The invention further provides methods for producing protein mol. weight ladders for use in protein gel electrophoresis, as well as proteins and protein mol. weight ladders produced by these methods.

REFERENCE COUNT: 16 THERE ARE 16 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS, LIFESCI' ENTERED AT 15:26:49 ON 15 FEB 2007

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L6        39309 S L3 AND L5
L7          738 S L6 AND INCLUSION
L8            2 S (BACTERIAL (W)HOST?) AND L7

=> s l7 and (molecular (w)weight)
L9           59 L7 AND (MOLECULAR (W) WEIGHT)

=> dup rem l9
PROCESSING COMPLETED FOR L9
L10          24 DUP REM L9 (35 DUPLICATES REMOVED)

=> d 1-24 ibib ab
```

L10 ANSWER 1 OF 24 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2006158471 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16289918
TITLE: Maize uroporphyrinogen III methyltransferase: overexpression of the functional gene fragments in Escherichia coli and one-step purification.
AUTHOR: Fan Jun; Wang Deqiang; Liang Zhi; Guo Min; Teng Maikun; Niu Liwen
CORPORATE SOURCE: Hefei National Laboratory of Physical Sciences at Microscale, Key Laboratory of Structural Biology of Chinese Academy of Science, 96 Jinzhai Road, Hefei, Anhui 230026, PR China.
SOURCE: Protein expression and purification, (2006 Mar) Vol. 46, No. 1, pp. 40-6. Electronic Publication: 2005-08-10. Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200605
ENTRY DATE: Entered STN: 22 Mar 2006
Last Updated on STN: 1 Jun 2006
Entered Medline: 31 May 2006

AB S-Adenosyl-L-methionine: uroporphyrinogen III methyltransferase (SUMT), a key regulatory enzyme, converts uroporphyrinogen III to precorrin-2 in the porphinoids biosynthesis. In this study, the mature SUMT was signified that the maize SUMT precursor encoded by the open reading frame of maize SUMT cDNA was deleted the first 91 amino acids constituting the postulated signal peptide. Several mature SUMT fusion and deletion mutants were conducted. It actively expressed in Escherichia coli that the mature SUMT, or the truncated one deleting the C-terminal extra 52 amino acids based on SUMT sequence comparisons. On the contrary, it expressed as an inclusion body in E. coli that the mature SUMT fusion mutant, the SUMT precursor, or the mature SUMT deleting the N-terminal 36 amino acids including glycine-rich region involved directly in SAM binding. The purified His6-tagged mature SUMT was homodimer with a molecular weight of 34 kDa, as shown by SDS-PAGE, 52 kDa using gel-filtration chromatography, and 79 kDa by dynamic light scattering assay. Red fluorescent compounds were associated with the recombinant mature SUMT which were identified as sirohydrochlorin and trimethylpyrrocorphin by spectroscopic analysis. This association slightly altered the protein secondary structure confirmed by circular dichroism assay.

L10 ANSWER 2 OF 24 EMBASE
ACCESSION NUMBER: 2006004716 EMBASE
TITLE: Construction of prokaryotic expression system of TGF-β1 epitope gene and identification of recombinant fusion protein immunity.
AUTHOR: Guo Y.-H.; Hao Z.-M.; Luo J.-Y.; Wang J.-H.
CORPORATE SOURCE: Dr. Y.-H. Guo, Department of Infectious Diseases, Xi'an Jiaotong University, Xi'an 710004, China. xiaoqing9759@sina.com
SOURCE: World Journal of Gastroenterology, (28 Oct 2005) Vol. 11, No. 40, pp. 6389-6394. .
Refs: 26
ISSN: 1007-9327 CODEN: WJGAF2
COUNTRY: China
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
048 Gastroenterology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 19 Jan 2006
Last Updated on STN: 19 Jan 2006

AB Aim: To insert the constructed TGF-β(1) epitope gene into the el loop of C-terminus of truncated hepatitis B core antigen to increase TGF-β(1) antigenicity in its prokaryotic expression system and to identify immunity of the expressed recombinant protein in order to exploit the possibility for obtaining anti-TGF-β(1) vaccine. Methods: The TGF-β(1) encoding epitope gene (the mature TGF-β(1) from 78-109 amino acid residues, TGF-β(1)(32)) was amplified by polymerase chain reaction from the recombinant pGEM-7z/. TGF-β (1)(32) vector. The HBcAg gene fragments (encoding HBcAg from 1-71 and 89-144 amino acid residues) were amplified from PYTA1-HBcAg vector. The recombinant vector pGEMEX-1 was used to insert HBcAg1-71, TGF-β(1)(32) and HBcAg89-144

into restrictive endonuclease enzyme and ligated with T(4) ligase. The fusion gene fragments HBcAg1-71-TGF-β(1) (32)-HBcAg89-144 were recloned to pET28a(+) and the DNA sequence was confirmed by the dideoxy chain termination method. The recombinant vector pET28a (+)/CTC was transformed and expressed in E. coli BL21 (DE3) under induction of IPTG. After purification with Ni(+2)-NTA agarose resins, the antigenicity of purified protein was detected by ELISA and Western blot and visualized under electron microscope. Results: Enzyme digestion analysis and sequencing showed that TGF-β1 epitope gene was inserted into the el loop of C-terminus of truncated hepatitis B core antigen. SDS-PAGE analysis showed that relative molecular mass (Mr) of the expressed product by pET28a (+)/CTC was Mr 24 600.The output of the target recombinant protein was approximately 34.8% of the total bacterial protein, mainly presented in the form of inclusion body. Western blotting and ELISA demonstrated that the fusion protein could combine with anti-TGF-β (1) polyclonal IgG but not with anti-HBcAg. The purity of protein was about 90 % and the protein was in the form of self-assembling particles visualized under electron microscope. This fusion protein had good anti-TGF-β(1) antigenicity and could be used as anti-TGF-β(1) vaccine. Conclusion: A recombinant prokaryotic expression system with high expression efficiency of the target TGF-β(1) epitope gene was successfully established. The fusion protein is in the form of self-assembling particles and HBcAg can increase the antigenicity of TGF-β(1). The expressed TGF-β1 epitope gene shows good immunogenicity and antigenicity.

L10 ANSWER 3 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
DUPLICATE 2

ACCESSION NUMBER: 2005:398937 BIOSIS
DOCUMENT NUMBER: PREV200510184176
TITLE: Expression, purification and biological activity of a recombinant human parathyroid hormone(1-34) analogue in E-coli.
AUTHOR(S): Di Xu; Liu Chang-Zheng; Chen Song-Sen [Reprint Author]; Zhang Yan-Li; Deng Yan-Chun; Sun Lan; Yang Jing
CORPORATE SOURCE: Chinese Acad Med Sci, Sch Basic Med, Peking Union Med Coll,Natl Lab Mol Biol, Inst Basic Med Sci, Beijing 100005, Peoples R China
chen-ss@263.net
SOURCE: Zhongguo Shengwu Huaxue yu Fenzi Shengwu Xuebao, (APR 20 2005) Vol. 21, No. 2, pp. 227-233.
ISSN: 1007-7626.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 5 Oct 2005
Last Updated on STN: 5 Oct 2005

AB Recombinant human parathyroid hormone (1-34) analogue with a amino-terminal extension of glycine, Gly-hPTH(1-34) was obtained from E. coli using a hydroxylamine cleavage fusion protein strategy. The DNA fragment encoding Asn-Gly-hPTH(1-34) was synthesized by overlapping PCR and cloned into the 3' end of the truncated GST gene(GST69 Delta) in expression vector pGEX-2T to construct the GST69 Delta and AsnGly-hPTH (1-34) fusion expression vector in which there was a hydroxylamine site between the two genes. The fusion protein was expressed in the inclusion bodies in E. coli JM109, and yield rate was about 20 % of the total bacteria proteins. The inclusion bodies were dissolved with 8. 0 mol/L urea-glycine buffer and subjected to site-specific cleavage with hydroxylamine. SDS-PAGE analysis showed that about 80 % the fusion protein was cleaved to GST69 Delta and Gly-hPTH (1-34) analogue. Over 98 % purity of recombinant

Gly-hPTH (1-34) was obtained through Sephadex G-50 and RP-HPLC purification procedures. N-terminal amino acids were determined by sequencing, isoelectric point(pI) determined by capillary electrophoresis and molecular weight measured by Maldi-Tof mass spectra. The results showed that 16 amino acid residues were the same as natural hPTH(1-34) except the first one was glycine, pI 8. 40, molecular weight 4177. The bioactivity indicated that Gly-hPTH(1-34) analogue could stimulate remarkable proliferation of human osteoblast cells(HOSTE85), increase intracellular collagen sythesis, enhance intracellular cAMP production and celluar alkaline phosphatase (ALP) activity. Recombinant Gly-PTH (1-34) analogue has almost same bioactivity and immunoactivity of the chemically sythetic hPTH(1-34) and an amino-terminal extension of glycine has no effect on bioactivity of PTH(1-34)

L10 ANSWER 4 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2004:704465 HCAPLUS
DOCUMENT NUMBER: 142:108066
TITLE: Expression of Truncated Region of S Genome Segment of Hantaan Virus in E. coli and Preliminary Use of This Recombinant Protein
AUTHOR(S): Wang, Bin; Zhao, Baihui; Qian, Dongmeng; Yan, Zhiyong
CORPORATE SOURCE: Medical College, Qingdao University, Qingdao, 266021, Peop. Rep. China
SOURCE: Zhongguo Shengwu Gongcheng Zazhi (2004), 24(1), 45-48
CODEN: ZSGZAW; ISSN: 1671-8135
PUBLISHER: Zhongguo Shengwu Gongcheng Zazhishe
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB Objective: Hantaan virus S gene truncated region (SA) was cloned and expressed, and it was used in rapid diagnosis of HFRS. Methods: SA was obtained by RT-PCR from peripheral blood of the patients with hemorrhagic fever with renal syndrome (HFRS) and cloned into GST fusion protein expression vector PGEX4T-2 by PCR and restriction digest methods, and the recombinant protein was examined by immunoblot with polycloned antibodies. Results: The mol. wt. of SA was 49000 kDa and mainly expressed as inclusion body in Escherichia coli. Western-blot analyses demonstrated that this recombinant protein presented the specific antigenicity. Conclusion: The prokaryotic expression of portions of N protein of this specific Hantaan virus could be used to generate readily an efficiency in diagnostic assays for HFRS.

L10 ANSWER 5 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2003:696300 HCAPLUS
DOCUMENT NUMBER: 139:244688
TITLE: Truncated recombinant major outer membrane protein antigen (r56) of Orientia tsutsugamushi strains karp, kato and gilliam for use as vaccines and for immunodiagnosis
INVENTOR(S): Ching, Wei-Mei; Kelly, Daryl J.; Dasch, Gregory A.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 25 pp., Cont.-in-part of U.S. 6,482,415.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003165523	A1	20030904	US 2002-120837	20020412

US 6482415	B1	20021119	US 1998-218425	19981222
US 2003185837	A1	20031002	US 2002-171520	20020614
US 6699674	B2	20040302		

PRIORITY APPLN. INFO.: US 1997-68732P P 19971224
US 1998-218425 A2 19981222
US 2001-283373P P 20010413

AB A recombinant, refolded non-fusion polypeptide expressed from a truncated r56 gene of the causative agent of scrub typhus, Orientia tsutsugamushi for the Karp, Kato and Gilliam strains has been produced. The invention is useful for detecting prior exposure to scrub typhus, screening for and/or identification of at least one infectious strain-similarity (i.e. a Karp-like, Kato-like or Gilliam-like strain) based on its strength of reaction toward a truncated protein and as a component in vaccine formulations and production of immune globulins for passive prophylaxis and immunity in subjects.

L10 ANSWER 6 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2003335425 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12868067
TITLE: Tumor necrosis factor-alpha-induced cell death in U373 cells overexpressing alpha-synuclein.
AUTHOR: Stefanova Nadia; Schanda Kathrin; Klimaschewski Lars; Poewe Werner; Wenning Gregor K; Reindl Markus
CORPORATE SOURCE: Department of Neurology, University of Innsbruck, Innsbruck, Austria.
SOURCE: Journal of neuroscience research, (2003 Aug 1) Vol. 73, No. 3, pp. 334-40.
Journal code: 7600111. ISSN: 0360-4012.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200309
ENTRY DATE: Entered STN: 18 Jul 2003
Last Updated on STN: 5 Sep 2003
Entered Medline: 4 Sep 2003

AB Intracellular alpha-synuclein inclusion formation in glial cells is frequently seen in Parkinson's disease and multiple system atrophy. Microglial activation in these neurodegenerative disorders suggests that neuroinflammatory responses might interact with alpha-synuclein and contribute to the pathogenesis of these disorders. To study the role of tumor necrosis factor-alpha (TNF-alpha), an important proinflammatory cytokine produced by microglia, on cells overexpressing alpha-synuclein we have used the astrocytoma cell line U373 engineered to express C-terminally truncated alpha-synuclein as a fusion protein with red or green fluorescent proteins. We demonstrate that alpha-synuclein overexpression augmented TNF-alpha-induced apoptotic cell death in U373 cells by induction of caspase activation. Furthermore, TNF-alpha exposure was associated with significant cytoskeletal changes characterized by altered inclusion composition with loss of cytoskeletal proteins and elevation of high-molecular-weight alpha-synuclein species. We conclude that alpha-synuclein overexpression significantly increases the vulnerability of U373 cells to apoptosis through TNF-alpha-mediated pathways.

L10 ANSWER 7 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2002454088 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12208962
TITLE: Identification and characterization of three immunodominant structural proteins of fowlpox virus.
AUTHOR: Boulanger Denise; Green Philip; Jones Brenda; Henriquet Gwenn; Hunt Lawrence G; Laidlaw Stephen M; Monaghan Paul;

Skinner Michael A
CORPORATE SOURCE: Compton Laboratory, Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, United Kingdom.
SOURCE: Journal of virology, (2002 Oct) Vol. 76, No. 19, pp. 9844-55.
Journal code: 0113724. ISSN: 0022-538X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200209
ENTRY DATE: Entered STN: 6 Sep 2002
Last Updated on STN: 1 Oct 2002
Entered Medline: 30 Sep 2002

AB Genes encoding fowlpox virus (FWPV) structural proteins have been identified mainly by sequence homology with those from vaccinia virus (VACV), but little is known about the encoded proteins. Production of monoclonal antibodies (MAbs) against Poxine and HP1-440 (Munich) clone FP9 allowed the identification of three immunodominant FWPV proteins: the 39-kDa core protein (encoded by FPV168, homologous to VACV A4L), a 30- and 35-kDa protein doublet, and an abundant 63-kDa protein. The 30- and 35-kDa proteins are nonglycosylated, antigenically related proteins present in the intracellular mature virus membrane and localizing closely with the viral factories. N-terminal sequencing identified the 35-kDa protein as encoded by FPV140 (the FWPV homolog of VACV H3L). The 63-kDa protein forms covalently linked dimers and oligomers. It remained mainly insoluble upon detergent treatment of purified virus but did not localize closely with the viral factory. N-terminal sequencing was unsuccessful, suggesting N-terminal blocking. CNBr digestion generated a peptide encoded by FPV191, predicted to encode one of two FWPV A-type inclusion (ATI) proteins. The characteristics of the 63-kDa protein were inconsistent with published observations on cowpox or VACV ATI proteins (it appears to be essential). The 63-kDa protein, however, shares characteristics with both VACV p4c virus occlusion and 14-kDa fusion proteins. Gene assignment at the poxvirus ATI locus (between VACV A24R and A28L) is complicated by sequence redundancies and variations, often due to deletions and multiple frameshift mutations. The identity of FPV191 in relation to genes at this locus is discussed.

L10 ANSWER 8 OF 24 EMBASE
ACCESSION NUMBER: 2002137630 EMBASE
TITLE: Description of a cellulose-binding domain and a linker sequence from Aspergillus fungi.
AUTHOR: Quentin M.; Ebbelaar M.; Derksen J.; Mariani C.; Van der Valk H.
CORPORATE SOURCE: M. Quentin, Dept. of Fibre and Paper Technology, ATO BV, PO Box 17, 6700 AA Wageningen, Netherlands.
m.g.e.quentin@ato.wag-ur.nl
SOURCE: Applied Microbiology and Biotechnology, (2002) Vol. 58, No. 5, pp. 658-662.
Refs: 22
ISSN: 0175-7598 CODEN: AMBIDG
COUNTRY: Germany
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 2 May 2002
Last Updated on STN: 2 May 2002

AB A family I cellulose-binding domain (CBD) and a serine- and threonine-rich linker peptide were cloned from the fungi Aspergillus japonicus and Aspergillus aculeatus. A glutathione S-transferase (GST)

fusion protein comprising GST and a peptide linker with the CBD fused to its C-terminus, was expressed in Escherichia coli. The renatured GST-CBD recovered from inclusion bodies had a molecular mass of 36.5 kDa which agrees with the 29 kDa of the GST plus the calculated 7.5 kDa of the linker with the CBD. The isolated GST-CBD protein adsorbed to both bacterial microcrystalline cellulose and carboxymethyl cellulose. Deletion of the linker peptide caused a decrease in cellulose adsorbance and a higher sensitivity to protease digestion.

L10 ANSWER 9 OF 24 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001027362 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10913140
TITLE: Changing the donor cofactor of bovine alpha 1, 3-galactosyltransferase by fusion with UDP-galactose 4-epimerase. More efficient biocatalysis for synthesis of alpha-Gal epitopes.
AUTHOR: Chen X; Liu Z; Wang J; Fang J; Fan H; Wang P G
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, Michigan 48202, USA.
CONTRACT NUMBER: AI44040 (NIAID)
SOURCE: The Journal of biological chemistry, (2000 Oct 13) Vol. 275, No. 41, pp. 31594-600.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 22 Mar 2001
Last Updated on STN: 22 Mar 2001
Entered Medline: 13 Nov 2000

AB Two fusion enzymes consisting of uridine diphosphogalactose 4-epimerase (UDP-galactose 4-epimerase, EC) and alpha1, 3-galactosyltransferase (EC) with an N-terminal His(6) tag and an intervening three-glycine linker were constructed by in-frame fusion of the Escherichia coli galE gene either to the 3' terminus (f1) or to the 5' terminus (f2) of a truncated bovine alpha1, 3-galactosyltransferase gene, respectively. Both fusion proteins were expressed in cell lysate as active, soluble forms as well as in inclusion bodies as improperly folded proteins. Both f1 and f2 were determined to be homodimers, based on a single band observed at about 67 kDa in SDS-polyacrylamide gel electrophoresis and on a single peak with a molecular mass around 140 kDa determined by gel filtration chromatography for each of the enzymes. Without altering the acceptor specificity of the transferase, the fusion with the epimerase changed the donor requirement of alpha1, 3-galactosyltransferase from UDP-galactose to UDP-glucose and decreased the cost for the synthesis of biomedically important Galalpha1,3Gal-terminated oligosaccharides by more than 40-fold. For enzymatic synthesis of Galalpha1,3Galbeta1,4Glc from UDP-glucose and lactose, the genetically fused enzymes f1 and f2 exhibited kinetic advantages with overall reaction rates that were 300 and 50%, respectively, higher than that of the system containing equal amounts of epimerase and galactosyltransferase. These results indicated that the active sites of the epimerase and the transferase in fusion enzymes were in proximity. The kinetic parameters suggested a random mechanism for the substrate binding of the alpha1, 3-galactosyltransferase. This work demonstrated a general approach that fusion of a glycosyltransferase with an epimerase can change the required but expensive sugar nucleotide to a less expensive one.

L10 ANSWER 10 OF 24 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN DUPLICATE 5
ACCESSION NUMBER: 2001:27336 BIOSIS

DOCUMENT NUMBER: PREV200100027336
TITLE: Expression of truncated HTNV nucleoprotein and analysis of antigenic epitope.
AUTHOR(S): Xue Xiao-ping [Reprint author]; Xu Zhi-kai [Reprint author]; Ma Wen-yu [Reprint author]
CORPORATE SOURCE: Dept. of Microbiology, Fourth Military Medical University, Xi'an, 710032, China
SOURCE: Virologica Sinica, (Sep., 2000) Vol. 15, No. 3, pp. 220-225. print.
ISSN: 1003-5125.
DOCUMENT TYPE: Article
LANGUAGE: Chinese
ENTRY DATE: Entered STN: 10 Jan 2001
Last Updated on STN: 12 Feb 2002

AB The expressing vector carring various truncated fragments of S gene of HTNV strain 76-118 was constructed and the vector efficiently expressed in E. coli. The result demonstrated that the GST-NP fusion proteins exist in the form of inclusion bodies in E. coli, the expressing amount accounted for 29-36% of the total proteins, and the molecular weights were 72 kD, 66 kD, 54 kD and 44 kD, respectively. Western blot showed that all of the four fusion proteins were positive stained with HPR-labeled anti-GST McAb 3C11, but only the 72 kD and 54 kD fusion proteins were positive stained with HRP-labeled anti-NP McAb 1A8. Four recombinant NP (rNP), which molecular weights were 44 kD, 40 kD, 26 kD and 16 kD respectively, were obtained by removing GST from purified GST-NP fusion proteins with thrombin. Mapping of antigenic epitope was done by 19 strains McAb. The result showed that 72 kD fusion protein could react with 13 strains McAb, which was same as authentic NP of HTNV. The truncated fusion protein (deleting aa 1-37 at N-terminal and aa 402-429 at c-terminal) expressed by S1.1 kb fragment and S 0.5 kb fragment (deleting aa 1-274 at N-terminal) did not react with all 19 strains McAb. The truncated fusion protein (deleting aa 275-429 at c-terminal) expressed by S 0.7 kb fragment reacted with 5 strains of group-specific McAb, which suggested that antigenic epitopes on NP of HTNV were located in the N-terminal of NP and distribution of group-specific and type-specific antigenic epitope showed some regional.

L10 ANSWER 11 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 2000:500898 HCAPLUS
DOCUMENT NUMBER: 134:158226
TITLE: Construction of His6-human TNFβ fusion expression plasmid and single-step purification of its product
AUTHOR(S): Xu, Jianfen; Zhou, Qing; Ma, Zhizhang; Yu, Jianfa; Hua, Ming; Ding, Renrui
CORPORATE SOURCE: Laboratory of Immuno-technology, College of Life Science, Zhejiang University, Hangzhou, 310012, Peop. Rep. China
SOURCE: Yaowu Shengwu Jishu (2000), 7(1), 1-5
CODEN: YSJIFO; ISSN: 1005-8915
PUBLISHER: Yaowu Shengwu Jishu Bianjibu
DOCUMENT TYPE: Journal
LANGUAGE: Chinese

AB In this research, a deletion of human TNFβ which lacking 23 N-terminal amino acid residues was cloned into over expression vector pET-28-C(+), a T71ac promoter-based fusion plasmid was constructed. Then the recombinant plasmid was transformed into E. coli BL21 (DE3). The protein (His6-TNFβ) expressed by induction of IPTG has a mol. wt. 20,500KD by SDS-PAGE. The expressed protein band constructed 25% of total bacteria protein. The product

induced was mainly as inclusion bodies (IBs). The His6-TNFβ purified by Ni2+-Sepharose 6B column under the denaturation after IBs were washed with 2mol/L urea buffer and dissolved in 7mol/L urea solution The result of purification showed that the product purity was more than 90 % and the recovery rate was about 90 %. The purified His6-TNFβ fusion protein was slowing diluted with the maturation buffer and dialyzed against 0.02mol/L PB buffer. The cytotoxic activity of the His6-TNFβ was about (0.5-1.0)x106U/mg. These results lay the foundation of production and further research of the hTNFb deletion.

L10 ANSWER 12 OF 24 MEDLINE on STN
ACCESSION NUMBER: 1999339376 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10413031
TITLE: Increased vulnerability of NFH-LacZ transgenic mouse to traumatic brain injury-induced behavioral deficits and cortical damage.
AUTHOR: Nakamura M; Saatman K E; Galvin J E; Scherbel U; Raghupathi R; Trojanowski J Q; McIntosh T K
CORPORATE SOURCE: Department of Neurosurgery, School of Medicine, University of Pennsylvania, Philadelphia 19104-6316, USA.
CONTRACT NUMBER: P01-NS08803 (NINDS)
R01-GM34690 (NIGMS)
R01-NS26818 (NINDS)
+
SOURCE: Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, (1999 Jul) Vol. 19, No. 7, pp. 762-70.
Journal code: 8112566. ISSN: 0271-678X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 13 Sep 1999
Last Updated on STN: 13 Sep 1999
Entered Medline: 2 Sep 1999

AB The authors evaluated the neurobehavioral and neuropathologic sequelae after traumatic brain injury (TBI) in transgenic (TG) mice expressing truncated high molecular weight neurofilament (NF) protein fused to beta-galactosidase (NFH-LacZ), which develop Lewy body-like NF-rich inclusions throughout the CNS. TG mice and their wild-type (WT) littermates were subjected to controlled cortical impact brain injury (TG, n = 19; WT, n = 17) or served as uninjured controls (TG, n = 11; WT, n = 11). During a 3-week period, mice were evaluated with an array of neuromotor function tests including neuroscore, beam balance, and both fast and slow acceleration rotarod. Brain-injured WT and TG mice showed significant motor dysfunction until 15 days and 21 days post-injury, respectively (P<.025). Compared with brain-injured WT mice, brain-injured TG mice had significantly greater motor dysfunction as assessed by neuroscore (P<.01) up to and including 15 days post-injury. Similarly, brain-injured TG mice performed significantly worse than brain-injured WT mice on slow acceleration rotarod at 2, 8, and 15 days post-injury (P<.05), and beam balance over 2 weeks post-injury (P<.01). Histopathologic analysis showed significantly greater tissue loss in the injured hemisphere in TG mice at 4 weeks post-injury (P<.01). Together these data show that NFH-LacZ TG mice are more behaviorally and histologically vulnerable to TBI than WT mice, suggesting that the presence of NF-rich inclusions may exacerbate neuromotor dysfunction and cell death after TBI.

L10 ANSWER 13 OF 24 MEDLINE on STN
ACCESSION NUMBER: 1998298057 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9632604

TITLE: A 20-kilodalton N-terminal fragment of the D15 protein contains a protective epitope(s) against Haemophilus influenzae type a and type b.
AUTHOR: Yang Y p; Thomas W R; Chong P; Loosmore S M; Klein M H
CORPORATE SOURCE: Research Center, Pasteur Merieux Connaught Canada, North York, Ontario, Canada M2R 3T4.. ypyang@ca.pmc-vacc.com
SOURCE: Infection and immunity, (1998 Jul) Vol. 66, No. 7, pp. 3349-54.
Journal code: 0246127. ISSN: 0019-9567.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 16 Jul 1998
Last Updated on STN: 16 Jul 1998
Entered Medline: 9 Jul 1998

AB A conserved 80-kDa minor outer membrane protein, D15, of Haemophilus influenzae has been shown to be a protective antigen in laboratory animals against H. influenzae type a (Hia) or type b (Hib) infection. To localize the protective B-cell epitope(s) within the D15 protein and to further explore the possibility of using synthetic peptides as vaccine antigens, a 20-kDa N-terminal fragment of D15 protein (truncated D15 [tD15]) was expressed as a fusion protein with glutathione S-transferase in Escherichia coli. The tD15 moiety was cleaved from glutathione S-transferase by using thrombin and purified to homogeneity. The purified soluble tD15 appeared to contain immunodominant protective epitope(s) against Hia and Hib, since rabbit antisera directed against tD15 were capable of protecting infant rats from Hia or Hib bacteremia. The ease of purification of soluble tD15, therefore, makes it a better candidate antigen than the full-length recombinant D15 which is produced as inclusion bodies in E. coli. Furthermore, both the purified tD15 fragment and a mixture of tD15-derived peptides spanning amino acid residues 93 to 209 of the mature D15 protein were capable of inhibiting the protection against Hib conferred on infant rats by rabbit anti-tD15 antiserum, indicating that the protective epitopes of D15 may not be conformational. However, the administration of pooled rabbit immune sera raised against the same panel of peptides failed to protect infant rats from Hib infection.

L10 ANSWER 14 OF 24 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 97178320 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8994061
TITLE: Selective degeneration fo Purkinje cells with Lewy body-like inclusions in aged NFHLACZ transgenic mice.
AUTHOR: Tu P H; Robinson K A; de Snoo F; Eyer J; Peterson A; Lee V M; Trojanowski J Q
CORPORATE SOURCE: Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia 19104, USA.
SOURCE: The Journal of neuroscience : the official journal of the Society for Neuroscience, (1997 Feb 1) Vol. 17, No. 3, pp. 1064-74.
Journal code: 8102140. ISSN: 0270-6474.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199703
ENTRY DATE: Entered STN: 27 Mar 1997
Last Updated on STN: 27 Mar 1997
Entered Medline: 17 Mar 1997

AB Transgenic (NFHLacZ) mice expressing a fusion protein

composed of a truncated high-molecular-weight mouse neurofilament (NF) protein (NFH) fused to beta-galactosidase (LacZ) develop inclusions in neurons throughout the CNS. These inclusions persist from birth to advanced age and contain massive filamentous aggregates including all three endogenous NF proteins and the NFHLacZ fusion protein. Further, the levels of endogenous NF proteins are selectively reduced in NFHLacZ mice. Because these inclusions resemble NF-rich Lewy bodies (LBs) in Parkinson's disease and LB dementia, we asked whether these lesions compromised the viability of affected neurons during aging. We studied hippocampal CA1 neurons, nearly all of which harbored inclusions (type I) devoid of cellular organelles, and cerebellar Purkinje cells, nearly all of which accumulated inclusions (type II) containing numerous entrapped organelles. Purkinje cells with type II inclusions began to degenerate in the NFHLacZ mice at approximately 1 year of age, and most were eliminated by 18 months of age. In contrast, there was no significant loss of type I inclusion-bearing CA1 neurons with age. These data suggest that the sequestration of cellular organelles in type II inclusions may isolate and impair the function of these organelles, thereby rendering Purkinje cells selectively vulnerable to degeneration with age as in neurodegenerative diseases of the elderly characterized by accumulation of LBs.

L10 ANSWER 15 OF 24 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 97110341 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8952502
TITLE: Reconstitution of the 2Fe-2S center and g = 1.89 electron paramagnetic resonance signal into overproduced Nostoc sp. PCC 7906 Rieske protein.
AUTHOR: Holton B; Wu X; Tsapin A I; Kramer D M; Malkin R; Kallas T
CORPORATE SOURCE: Department of Biology and Microbiology, University of Wisconsin, Oshkosh 54901, USA.
CONTRACT NUMBER: GM-20571 (NIGMS)
SOURCE: Biochemistry, (1996 Dec 3) Vol. 35, No. 48, pp. 15485-93. Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 29 Jan 1999
Entered Medline: 9 Jan 1997

AB The Rieske 2Fe-2S protein is a distinguishing subunit of the photosynthetic electron transport cytochrome b6f complex in chloroplast and cyanobacterial thylakoid membranes. We have constructed plasmids for overproduction in Escherichia coli of fusion, full-length, and truncated forms of the Rieske (PetC) protein from the cyanobacterium Nostoc sp. PCC 7906. A glutathione S-transferase/Rieske fusion protein was used to prepare specific chicken egg-yolk antibodies against the Rieske protein. Expression of the nonfusion petC gene in a T7 RNA polymerase promoter vector produced copious quantities of the full-length Rieske protein predominantly as inclusion bodies. The highly enriched, Rieske protein from inclusion bodies has been denatured in guanidine hydrochloride and refolded and the characteristic 2Fe-2S cluster reconstituted in vitro by incubation with iron and sulfide under reducing conditions. Purification by chromatography on Whatman DE52 cellulose and ultrafiltration through a 30000 molecular weight cutoff membrane yielded pure and predominantly monomeric Rieske protein. Reconstituted Rieske preparations showed intense and highly characteristic gx = 1.74, gy = 1.89, and gz = 2.03 "Rieske-type" electron paramagnetic resonance signals at 15 K. Two methods of reconstitution yielded Rieske preparations in which 20-60% of the protein contained 2Fe-2S clusters as determined by EPR

spin quantitation. The reconstituted Rieske protein was soluble and stable at 4 degrees C in buffers containing nonionic detergents and showed a redox midpoint potential of +321 mV at pH 7.0 as determined by optical circular dichroism (CD) spectroscopy. These data demonstrate the in vitro restoration of a Cys and His liganded 2Fe-2S cluster and provide the basis for mutational and structural analysis of a PetC Rieske protein of oxygenic photosynthesis.

L10 ANSWER 16 OF 24 MEDLINE on STN
ACCESSION NUMBER: 96410777 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8812850
TITLE: Expression of the extracellular domain of the human heat-stable enterotoxin receptor in Escherichia coli and generation of neutralizing antibodies.
AUTHOR: Nandi A; Mathew R; Visweswariah S S
CORPORATE SOURCE: Center for Reproductive Biology and Molecular Endocrinology, Indian Institute of Science, Bangalore, 560012, India.
SOURCE: Protein expression and purification, (1996 Sep) Vol. 8, No. 2, pp. 151-9.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199612
ENTRY DATE: Entered STN: 28 Jan 1997
Last Updated on STN: 28 Jan 1997
Entered Medline: 3 Dec 1996

AB The entire extracellular domain of the human heat-stable enterotoxin (ST) receptor as well as a truncated N-terminal domain were cloned as glutathione S-transferase fusion proteins and expressed in Escherichia coli. The recombinant fusion proteins were purified from both the cytosol and the inclusion body fractions by selective detergent extraction followed by glutathione-agarose affinity chromatography. The purified protein, corresponding to the entire extracellular domain, bound the stable toxin peptide with an affinity comparable to that of the native receptor characterized from the human colonic T84 cell line. No binding was observed with the N-terminal truncated fragment of the receptor under similar conditions. Polyclonal antibodies were raised to the entire extracellular domain fusion protein as well as the truncated extracellular domain fusion protein, and the antibodies were purified by affinity chromatography. Addition of the purified antibodies to T84 cells inhibited ST binding and abolished ST-mediated cGMP production, indicating that critical epitopes involved in ligand interaction are present in the N-terminal fragment of the receptor. Purified antibodies recognized a single protein of Mr 160,000 Da on Western blotting with T84 membranes, corresponding to a size of the native glycosylated receptor in T84 cells. These studies are the first report of the expression, purification, and characterization of any member of the guanylyl cyclase family of receptors in E. coli and show that binding of the toxin to the extracellular domain of the receptor is possible in the absence of any posttranslational modifications such as glycosylation. The recombinant fusion proteins as well as the antibodies that we have generated could serve as useful tools in the identification of critical residues of the extracellular domain involved in ligand interaction.

L10 ANSWER 17 OF 24 MEDLINE on STN
ACCESSION NUMBER: 96207400 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8615013
TITLE: Analysis of HPV1 E4 complexes and their association with keratins in vivo.

AUTHOR: Doorbar J; Medcalf E; Napthine S
CORPORATE SOURCE: Division of Virology, Department of Pathology, Cambridge University, Tennis Court Road, Cambridge, CB2 1QP, England.
SOURCE: Virology, (1996 Apr 1) Vol. 218, No. 1, pp. 114-26.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199606
ENTRY DATE: Entered STN: 13 Jun 1996
Last Updated on STN: 9 Feb 2000
Entered Medline: 5 Jun 1996

AB The HPV1 E4 gene encodes a family of abundant nonstructural late proteins whose role in the virus life cycle is unknown. Their localization to keratin filaments when expressed in cultured epithelial cells has suggested a possible involvement in virus release by disturbing the integrity of the infected cell. Here we show that in naturally occurring HPV1-induced tumors, the majority of the E4 protein (>95%) exists as complexes which do not contain keratins. The identification of discrete species of 34K, 58K, 70K, 88K, and 105K suggests that these are simple multimers of the 17K monomer, with very little of the soluble E4 being present in complexes larger than 140K. The truncated 10/11K E4 species, which comprise the C-terminal domain of E4, exist as trimers and dimers in vivo. Less than 5% of the E4 was present as complexes greater than 140K, and these were found to be insoluble. The 34K (dimer) and 58K (putative trimer) E4 complexes were components of these larger structures. Neither E4 monomers nor E4 complexes could be shown to interact directly with keratins or with keratin filaments although formation of the 105K E4 complex was abolished (and formation of the 58K species enhanced) when keratins were present during E4 synthesis in vitro. We conclude that while E1-E4 may transiently associate with keratins during synthesis, the two proteins do not stably associate via a direct interaction. The majority of the HPV1 E4 protein in established tumors in vivo is neither filament associated nor contained in inclusion granules, but exists predominantly as soluble cytoplasmic multimers.

L10 ANSWER 18 OF 24 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 95399776 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7670112
TITLE: A murine cytokine fusion toxin specifically targeting the murine granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor on normal committed bone marrow progenitor cells and GM-CSF-dependent tumor cells.
AUTHOR: Chan C H; Blazar B R; Eide C R; Kreitman R J; Vallera D A
CORPORATE SOURCE: Department of Therapeutic Radiology, University of Minnesota Hospital and Clinics, Minneapolis 55455, USA.
CONTRACT NUMBER: R01-CA31618 (NCI)
R01-CA36725 (NCI)
SOURCE: Blood, (1995 Oct 1) Vol. 86, No. 7, pp. 2732-40.
Journal code: 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199510
ENTRY DATE: Entered STN: 26 Oct 1995
Last Updated on STN: 26 Oct 1995
Entered Medline: 19 Oct 1995

AB A fusion protein was synthesized consisting of the murine granulocyte-macrophage colony-stimulating factor (mGM-CSF) gene spliced to a truncated form of the diphtheria toxin (DT390) gene coding for a molecule that retained full enzymatic activity, but excluded the native

binding domain. The DT390-mGM-CSF hybrid gene was cloned into a vector under the control of an inducible promoter and the protein expressed in Escherichia coli. After induction, a protein was purified from inclusion bodies in accord with the deduced molecular weight of DT390 mGM-CSF. Cell-free studies of the adenosine diphosphate-ribosylating activity of DT390 mGM-CSF showed results that were similar to those of native DT. The DT390 mGM-CSF immunotoxin inhibited FDCP2.1d, a murine myelomonocytic tumor line expressing the GM-CSF receptor with an IC50 (concentration inhibiting 50% activity) of 5 x 10(-11) mol/L. The fusion toxin was specifically cytotoxic and directed by the GM-CSF portion of the molecule because addition of a monoclonal antibody directed against GM-CSF inhibited its ability to kill the cell line. Cell lines that do not express GM-CSF receptor were not inhibited by the fusion toxin. DT390 mGM-CSF was also able to specifically inhibit normal committed bone marrow (BM) progenitor cells as measured in clonal colony-forming unit granulocyte-macrophage assays. Together, these findings indicate that DT390 mGM-CSF may be useful as a novel tool for purging BM of contaminating leukemia cells or in vivo for eliminating residual leukemia cells. Also, it can be used to determine whether committed and/or noncommitted BM progenitor cells express the GM-CSF receptor.

L10 ANSWER 19 OF 24 EMBASE

ACCESSION NUMBER: 95269173 EMBASE
DOCUMENT NUMBER: 1995269173
TITLE: Recombinant soluble human tissue factor secreted by Saccharomyces cerevisiae and refolded from Escherichia coli inclusion bodies: Glycosylation of mutants, activity and physical characterization.
AUTHOR: Stone M.J.; Ruf W.; Miles D.J.; Edgington T.S.; Wright P.E.
CORPORATE SOURCE: Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road,La Jolla, CA 92037, United States
SOURCE: Biochemical Journal, (1995) Vol. 310, No. 2, pp. 605-614. ISSN: 0264-6021 CODEN: BIJOAK
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 26 Sep 1995
Last Updated on STN: 26 Sep 1995

AB Tissue factor (TF) is the cell-surface transmembrane receptor that initiates both the extrinsic and intrinsic blood coagulation cascades. The abilities of TF to associate with Factor VIIa and Factor X in a ternary complex and to enable proteolytic activation of Factor X by Factor VIIa reside in the extracellular domain of TF. We describe the expression of the surface domain of TF (truncated TF, tTF) in both Saccharomyces cerevisiae and Escherichia coli and the biochemical and physical characterization of the recombinant proteins. Wild-type tTF and several glycosylation-site mutants were secreted efficiently by S. cerevisiae under the control of the yeast prepro-α-signal sequence; the T13A,N137D double mutant was the most homogeneous variant expressed in milligram quantities. Wild-type tTF was expressed in a non-native state in E. coli inclusion bodies as a fusion protein with a poly(His) leader. The fusion protein could be fully renatured and the leader removed by proteolysis with thrombin; the correct molecular mass (24 729 Da) of the purified protein was confirmed by electrospray mass spectrometry. Recombinant tTFs from yeast, E. coli and Chinese hamster ovary cells were identical in their abilities to bind Factor VIIa, to enhance the catalytic activity of Factor VIIa and to enhance the

proteolytic activation of Factor X by Factor VIIa. Furthermore, CD, fluorescence emission and NMR spectra of the yeast and E. coli proteins indicated that these proteins are essentially identical structurally.

L10 ANSWER 20 OF 24 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 95313353 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7793070
TITLE: Expression and purification of a recombinant tobacco etch virus NIa proteinase: biochemical analyses of the full-length and a naturally occurring truncated proteinase form.
AUTHOR: Parks T D; Howard E D; Wolpert T J; Arp D J; Dougherty W G
CORPORATE SOURCE: Department of Microbiology, Oregon State University, Corvallis 97331-3804, USA.
SOURCE: Virology, (1995 Jun 20) Vol. 210, No. 1, pp. 194-201.
Journal code: 0110674. ISSN: 0042-6822.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199507
ENTRY DATE: Entered STN: 7 Aug 1995
Last Updated on STN: 3 Feb 1997
Entered Medline: 24 Jul 1995

AB The tobacco etch virus 27-kDa nuclear inclusion a (NIa) proteinase was expressed in Escherichia coli as a recombinant fusion protein containing a seven-histidine tag at the amino-terminus. Catalytically active and inactive (by virtue of a single amino acid change) forms of the proteinase were purified to homogeneity in a two-column chromatographic procedure. The active form of the proteinase was slowly converted to a lower molecular weight form, while the inactive form was not. This conversion was dilution independent and thought to be intramolecular. Isolation of the approximately 2-kDa peptide cleavage product and determination of its N-terminal amino acid sequence positioned the cleavage site 24 amino acids from the carboxy-terminus of the proteinase. A recombinant NIa proteinase lacking the C-terminal 24 amino acids was shown to possess limited activity. Kinetic analyses of cleavage of a synthetic peptide by the full-length or truncated proteinase were conducted and indicated that the Km of the truncated proteinase was approximately fourfold higher than that of the full-length form. The truncated proteinase was approximately one-twentieth as efficient in proteolysis of the test peptide substrate as the full-length form.

L10 ANSWER 21 OF 24 MEDLINE on STN
ACCESSION NUMBER: 95091522 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7998836
TITLE: Expression and characterisation of the influenza A virus non-structural protein NS1 in yeast.
AUTHOR: Ward A C; Azad A A; Macreadie I G
CORPORATE SOURCE: Biomolecular Research Institute, Parkville, Victoria, Australia.
SOURCE: Archives of virology, (1994) Vol. 138, No. 3-4, pp. 299-314.
Journal code: 7506870. ISSN: 0304-8608.
PUB. COUNTRY: Austria
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-L25833
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 26 Jan 1995
Last Updated on STN: 3 Mar 2000
Entered Medline: 13 Jan 1995

AB The influenza A virus non-structural protein NS1 was produced using a copper-inducible expression system in the yeast Saccharomyces cerevisiae. The protein produced had a molecular weight of 26 kDa by SDS-PAGE and was reactive with anti-NS1 antisera. The recombinant NS1 protein was targetted to the nucleolus/nuclear envelope fraction of the yeast cell nucleus, showing that its localisation signals remain functional in yeast. In addition, immune-electron microscopy detected cytoplasmic inclusions reminiscent of those seen in cells infected with some influenza strains. The NS1 protein was shown to be capable of in vivo self-interaction which probably forms the basis of its propensity to form inclusions. Expression of the protein was found to be toxic to yeast cells expressing it, supporting a role for the protein in the shutdown of influenza virus-infected cells. Deletion mapping of NS1 pointed to 2 regions of the molecule being important for this toxicity: a basic C-terminal stretch which has been shown to act as a nuclear localisation signal, and an N-terminal region implicated in RNA binding.

L10 ANSWER 22 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1994:29229 HCAPLUS
DOCUMENT NUMBER: 120:29229
TITLE: Antibody recognition of the recombinant human nuclear antigens RNP 70 kD, SS-A, SS-B, Sm-B and Sm-D by autoimmune sera
AUTHOR(S): Wagatsuma, Masako; Asami, Noriko; Miyachi, Junko; Uchida, Sanae; Watanabe, Hiroshi; Amann, Egon
CORPORATE SOURCE: Pharma Res. Lab., Hoechst Japan Ltd., Kawagoe, 350, Japan
SOURCE: Molecular Immunology (1993), 30(16), 1491-8
CODEN: MOIMD5; ISSN: 0161-5890
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Five human nuclear antigens, RNP 70 kDa, SS-A, SS-B, Sm-B and Sm-D, were produced in E. coli using the expression vector pSEM. The cDNAs encoding these antigens were ligated to a truncated lacZ' gene of the vector and the β-galactosidase fusion proteins were efficiently expressed as intracellular inclusion bodies after isopropyl-β-thiogalactopyranoside induction. The antibody reactivities of these fusion proteins were evaluated by Western blot and by ELISA employing panel sera from patients with autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome or mixed connective tissue disease. The three fusion proteins, RNP 70 kDa, SS-B, and Sm-B, showed good reactivities in both systems, whereas the other two fusion proteins, SS-A and Sm-D, showed poor and no reactivity in both systems, whereas the other two fusion proteins, SS-A and Sm-D, showed poor and no reactivity in both systems, resp. It can be concluded that RNP 70 kDa, SS-B and Sm-B recombinant antigens are useful reagents for the differential diagnosis of the autoimmune diseases.

L10 ANSWER 23 OF 24 HCAPLUS COPYRIGHT 2007 ACS on STN
ACCESSION NUMBER: 1993:184979 HCAPLUS
DOCUMENT NUMBER: 118:184979
TITLE: Novel DNA binding proteins participate in the regulation of human neurofilament H gene expression
AUTHOR(S): Elder, Gregory A.; Liang, Zuozong; Lee, Nancy; Friedrich, Victor L., Jr.; Lazzarini, Robert A.
CORPORATE SOURCE: Brookdale Cent. Mol. Biol., Mt. Sinai Sch. Med., New York, NY, 10029, USA
SOURCE: Molecular Brain Research (1992), 15(1-2), 85-98
CODEN: MBREE4; ISSN: 0169-328X
DOCUMENT TYPE: Journal
LANGUAGE: English

AB By a combination of DNase I footprinting, methylation interference, and gel shift analyses the authors have identified multiple binding sites for nuclear proteins within the promoter region of the human neurofilament H gene. Two sites likely bind the transcription factor Sp1 while two others may be targets for previously unrecognized DNA binding proteins. One site, PAL, occurs within the 10 bp sequence GGGGAGGAGG. Two copies of the PAL sequence form an interrupted palindrome around one of the Sp1 sites. A second site, PROX, is found within the sequence GGTTGGACC. Nuclear exts. prepared from both neural and non-neural cell lines, mouse brain, and mouse liver contain proteins that recognize and bind to the PROX and PAL sequences indicating that proteins which bind to these target sequences are widespread. The appearance of these target sequences in the 5' upstream region of several neuron specific genes suggests that they play key roles in the transcription of neuron specific genes. The functional activity of these target DNA sequences was demonstrated by transfection assays using a reporter gene fused to nested deletions of the NF(H) promoter region. Interestingly, these assays revealed that maximal transient expression was obtained with DNA fusion genes containing the PAL, PROX and TATA sequences. Inclusion of the Sp1 sites into the fusion genes failed to enhance the expression of the reporter gene. To determine if the NF(H) promoter can be activated in a tissue specific manner during development transgenic mice containing the promoter region linked to a β-galactosidase reporter gene were generated. In one line sporadic expression of the transgene occurred in the CNS and testis while in four other lines no expression occurred. Collectively these results suggest that the NF(H) gene promoter is active in a tissue specific manner only by interactions with regulatory elements that lie further upstream or downstream of the start site of initiation.

L10 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 90386769 MEDLINE
DOCUMENT NUMBER: PubMed ID: 1366560
TITLE: High-level expression of human insulin-like growth factor II in Escherichia coli.
AUTHOR: Rhee H J; Lee Y I; Yang K H
CORPORATE SOURCE: Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology, Seoul.
SOURCE: Journal of biotechnology, (1990 Mar) Vol. 13, No. 4, pp. 293-304.
Journal code: 8411927. ISSN: 0168-1656.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Biotechnology
ENTRY MONTH: 199007
ENTRY DATE: Entered STN: 9 Aug 1995
Last Updated on STN: 9 Aug 1995
Entered Medline: 20 Jul 1990

AB A gene encoding mature human insulin-like growth factor II (IGF-II) was constructed from the modified IGF-II cDNA sequence and two double-stranded synthetic oligodeoxynucleotide linkers. It was fused to a truncated lacZ gene such that IGF-II was expressed as part of C-terminus of beta-galactosidase. This fused lacZ'-IGF-II gene was under the control of tac promoter and we overproduced the beta-galactosidase-IGF-II fusion protein in the Escherichia coli. The fusion protein formed inclusion bodies inside the cells. The fusion protein was purified from the isolated inclusion bodies and IGF-II protein was obtained from their fusion protein by CNBr cleavage. The released IGF-II was confirmed by its molecular weight as determined by SDS-PAGE and by its ability to bind anti-IGF antibody.

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=> e chatterjee d/au
E1          19      CHATTERJEE CHIRADIP/AU
E2          46      CHATTERJEE CHITRALEKHA/AU
E3        1115 --> CHATTERJEE D/AU
E4           2      CHATTERJEE D */AU
E5           1      CHATTERJEE D B/AU
E6           6      CHATTERJEE D C/AU
E7           5      CHATTERJEE D D/AU
E8           1      CHATTERJEE D E B/AU
E9          36      CHATTERJEE D J/AU
E10        386      CHATTERJEE D K/AU
E11         54      CHATTERJEE D N/AU
E12         27      CHATTERJEE D P/AU

=> s e3
L11       1115 "CHATTERJEE D"/AU

=> e longo m/au
E1          19      LONGO LUIGIA/AU
E2           6      LONGO LUIZ S JR/AU
E3         739 --> LONGO M/AU
E4         119      LONGO M A/AU
E5           9      LONGO M B/AU
E6          27      LONGO M C/AU
E7           2      LONGO M CASTELLANI/AU
E8          15      LONGO M D/AU
E9           2      LONGO M D C/AU
E10         20      LONGO M F/AU
E11         20      LONGO M G/AU
E12         15      LONGO M I/AU

=> s e3
L12        739 "LONGO M"/AU

=> e flynn e/au
E1           1      FLYNN DUANE J/AU
E2           3      FLYNN DUSTIE/AU
E3         291 --> FLYNN E/AU
E4          57      FLYNN E A/AU
E5           2      FLYNN E B/AU
E6           5      FLYNN E C/AU
E7           9      FLYNN E D/AU
E8           3      FLYNN E E/AU
E9           1      FLYNN E F/AU
E10         11      FLYNN E H/AU
E11        152      FLYNN E J/AU
E12          1      FLYNN E J */AU

=> s e3
L13        291 "FLYNN E"/AU

=> e oberfelder r/au
E1           1      OBERFELD SHELDON M/AU
E2           1      OBERFELDARZT/AU
E3          14 --> OBERFELDER R/AU
E4          30      OBERFELDER R W/AU
E5           6      OBERFELDER ROBERT/AU
E6           5      OBERFELDER ROBERT W/AU
E7           1      OBERFELDER ROBERT WILLIAM/AU
E8          15      OBERFELL C R/AU
E9          10      OBERFELL CHARLES R/AU
E10          1      OBERFELL CHAS R/AU
E11         73      OBERFELL G G/AU
E12          1      OBERFELL G S/AU
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=> s e3-e7
L14          56 ("OBERFELDER R"/AU OR "OBERFELDER R W"/AU OR "OBERFELDER ROBERT"
                /AU OR "OBERFELDER ROBERT W"/AU OR "OBERFELDER ROBERT WILLIAM"/A
               U)

=> d his

     (FILE 'HOME' ENTERED AT 15:26:25 ON 15 FEB 2007)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 15:26:49 ON 15 FEB 2007
L1         28644 S THIOREDOXIN
L2        845647 S FUSION OR (PRE(W) STAINED (3W)LADDER) AND L1
L3         50640 S (TRUNCATED OR DELETION) AND L2
L4             3 S INCLUSION (W)PARTNER?
L5       8176504 S CLON? OR EXPRESS? OR RECOMBINANT
L6         39309 S L3 AND L5
L7           738 S L6 AND INCLUSION
L8             2 S (BACTERIAL (W)HOST?) AND L7
L9            59 S L7 AND (MOLECULAR (W)WEIGHT)
L10           24 DUP REM L9 (35 DUPLICATES REMOVED)
                 E CHATTERJEE D/AU
L11         1115 S E3
                 E LONGO M/AU
L12          739 S E3
                 E FLYNN E/AU
L13          291 S E3
                 E OBERFELDER R/AU
L14           56 S E3-E7

=> s l11 or l12 or l13 or l14
L15        2194 L11 OR L12 OR L13 OR L14

=> s l7 and l15
L16           0 L7 AND L15

=> s l1 and l15
L17           4 L1 AND L15

=> dup rem l17
PROCESSING COMPLETED FOR L17
L18           3 DUP REM L17 (1 DUPLICATE REMOVED)

=> d 1-3 ibib ab

L18  ANSWER 1 OF 3  BIOSIS  COPYRIGHT (c) 2007 The Thomson Corporation  on STN
ACCESSION NUMBER:   2004:219988  BIOSIS
DOCUMENT NUMBER:    PREV200400223055
TITLE:              Methods for production of proteins.
AUTHOR(S):          Chatterjee, Deb [Inventor, Reprint Author]; Longo, Mary
                    [Inventor]; Flynn, Elizabeth [Inventor]; Oberfelder,
                    Robert [Inventor]
CORPORATE SOURCE:   North Potomac, MD, USA
                    ASSIGNEE: Invitrogen Corporation, Frankfurt am Main, DE,
                    USA
PATENT INFORMATION: US 6703484 20040309
SOURCE:             Official Gazette of the United States Patent and Trademark
                    Office Patents, (Mar 9 2004) Vol. 1280, No. 2.
                    http://www.uspto.gov/web/menu/patdata.html. e-file.
                    ISSN: 0098-1133 (ISSN print).
DOCUMENT TYPE:      Patent
LANGUAGE:           English
ENTRY DATE:         Entered STN: 21 Apr 2004
```

Last Updated on STN: 21 Apr 2004

AB The current invention provides methods for producing a polypeptide as inclusion bodies in bacterial host cells. The present methods are carried out by forming a gene construct comprising the genetic sequence encoding a polypeptide operatively linked to that of an inclusion partner protein, such as E. coli thioredoxin or a modified E. coli thioredoxin, such that host cells comprising the gene construct produce the polypeptide as intracellular inclusion bodies. The methods of the present invention facilitate the rapid isolation and purification of recombinant proteins. In addition, the present methods may be useful for producing polypeptides or proteins which are small and are typically difficult to express, as well as those proteins that are toxic to host cells such as E. coli. The present invention also provides plasmids, vectors and host cells to be used in the present invention for production of polypeptides, and methods of production of polypeptides using these vectors and host cells. The invention further provides methods for producing protein molecular weight ladders for use in protein gel electrophoresis, as well as proteins and protein molecular weight ladders produced by these methods.

L18 ANSWER 2 OF 3 BIOTECHDS COPYRIGHT 2007 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 1998-09126 BIOTECHDS
TITLE: Production of polypeptides as inclusion bodies; recombinant protein preparation by plasmid pTrc99A or plasmid pTrxfus vector-mediated thioredoxin expression in Escherichia coli inclusion body
AUTHOR: Chatterjee D; Longo M; Flynn E; Oberfelder R
PATENT ASSIGNEE: Life-Technol.
LOCATION: Rockville, MD, USA.
PATENT INFO: WO 9830684 16 Jul 1998
APPLICATION INFO: WO 1998-US492 8 Jan 1998
PRIORITY INFO: US 1997-34658 8 Jan 1997
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1998-399134 [34]

AB A new method for the preparation of a protein in the form of an inclusion body involves: obtaining a host cell (e.g. Escherichia coli) containing a first DNA sequence encoding the protein linked to a second DNA sequence encoding an inclusion partner (thioredoxin or modified thioredoxin), forming a gene fusion construct (plasmid pTrcpr1-monomer or pTrxA-concat); and culturing the cell to favor production of the protein as inclusion bodies. Also new are: vector plasmid pTrc99A and plasmid pTrxfus containing the construct; a host cell containing the vector; making a protein mol.weight ladder composition by obtaining one or more DNA sequences encoding proteins of different mol.weight values, transforming host cells with the DNA, culturing the cells to favor production of each protein, and isolating each protein; and making one or more stained proteins by incubating the proteins with one or more protein-binding dyes under incubation conditions to complex the proteins with the dyes. The methods may be used to prepare a fragment of the gene-32 protein of phage T4, a fragment of KpnI-methylase or a fragment of E. coli Dead-Box protein or thioredoxin. (84pp)

L18 ANSWER 3 OF 3 BIOSIS COPYRIGHT (c) 2007 The Thomson Corporation on STN
ACCESSION NUMBER: 1997:421271 BIOSIS
DOCUMENT NUMBER: PREV199799720474
TITLE: Protein expression by inclusion.
AUTHOR(S): Oberfelder, R. W.; Flynn, E.; Chatterjee, D.
SOURCE: FASEB Journal, (1997) Vol. 11, No. 9, pp. A1200. Meeting Info.: 17th International Congress of Biochemistry and Molecular Biology in conjunction with the Annual

Meeting of the American Society for Biochemistry and Molecular Biology. San Francisco, California, USA. August 24-29, 1997.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 8 Oct 1997
Last Updated on STN: 8 Oct 1997

	Issue Date	Pages	Document ID	Title
1	20061221	30	US 20060286633 A1	Methods for production of proteins
2	20061130	30	US 20060269992 A1	Methods of production of proteins
3	20041014	31	US 20040204563 A1	Methods for production of proteins
4	20020530	35	US 20020065392 A1	METHODS FOR PRODUCTION OF PROTEIN
5	20040309	29	US 6703484 B2	Methods for production of proteins

	Issue Date	Pages	Document ID	Title
1	20061221	30	US 20060286633 A1	Methods for production of proteins
2	20061130	30	US 20060269992 A1	Methods of production of proteins
3	20060914	138	US 20060204524 A1	Genetically engineered clostridial genes, proteins encoded by the engineered genes, and uses thereof
4	20060727	642	US 20060165716 A1	Immunogenic compositions for gram positive bacteria such as streptococcus agalactiae
5	20050519	34	US 20050106605 A1	Homogeneous populations of molecules
6	20041014	31	US 20040204563 A1	Methods for production of proteins
7	20040729	69	US 20040146864 A9	Joining DNA sequences using topoisomerase I
8	20031127	66	US 20030219878 A1	Sticky rice
9	20031127	68	US 20030219773 A1	Joining DNA sequences using topoisomerase I
10	20020530	35	US 20020065392 A1	METHODS FOR PRODUCTION OF PROTEIN
11	20040309	29	US 6703484 B2	Methods for production of proteins
12	20031014	178	US 6632981 B1	DNA sequences encoding polypeptides having beta-1,3-glucanase activity

	Issue Date	Pages	Document ID	Title
13	20010717	177	US 6262342 B1	DNA sequences encoding polypeptides having .beta.-1,3-glucanase activity
14	19990824	181	US 5942662 A	Inducible herbicide resistance
15	19990309	172	US 5880328 A	DNA encoding plant chitinases
16	19990105	174	US 5856154 A	Method of protecting plants from oomycete pathogens
17	19981222	175	US 5851766 A	Process for isolating chemically regulatable DNA sequences
18	19981208	172	US 5847258 A	DNA encoding .beta.-1,3-glucanases
19	19980908	174	US 5804693 A	Chemically regulatable and anti-pathogenic DNA sequences and uses thereof
20	19980804	174	US 5789214 A	Method of inducing gene transcription in a plant
21	19980707	175	US 5777200 A	Chemically regulatable and anti-pathogenic DNA sequences and uses thereof
22	19980616	178	US 5767369 A	DNA sequences encoding SAR8.2 proteins and uses thereof
23	19971118	174	US 5689044 A	Chemically inducible promoter of a plant PR-1 gene
24	19970805	175	US 5654414 A	Chemically inducible promoter of a cucumber chitinase/lysozyme gene

	Issue Date	Pages	Document ID	Title
25	19970722	176	US 5650505 A	Chemically regulatable and anti-pathogenic DNA sequences and uses thereof
26	19970325	176	US 5614395 A	Chemically regulatable and anti-pathogenic DNA sequences and uses thereof

	Issue Date	Pages	Document ID	Title
1	20061221	30	US 20060286633 A1	Methods for production of proteins
2	20061130	30	US 20060269992 A1	Methods of production of proteins
3	20060914	138	US 20060204524 A1	Genetically engineered clostridial genes, proteins encoded by the engineered genes, and uses thereof
4	20050519	34	US 20050106605 A1	Homogeneous populations of molecules
5	20041014	31	US 20040204563 A1	Methods for production of proteins
6	20020530	35	US 20020065392 A1	METHODS FOR PRODUCTION OF PROTEIN
7	20040309	29	US 6703484 B2	Methods for production of proteins

	L #	Hits	Search Text
1	L1	1	"6703484".pn.
2	L2	6892	thioredoxin
3	L4	0	l2 same l3
4	L3	5	prestained adj ladder$2
5	L5	155	molecular adj weight adj ladder
6	L6	26	l2 and l5
7	L7	35620	LONGO FLYNN CHATTERJEE OBERFELDER
8	L8	7	l6 and l7

	U	1	Issue Date	Pages	Document ID	Title	Current OR	Current XRef	Retrieval Classif
1			20010130	13	US 6179872 B1	Biopolymer matt for use in tissue repair and reconstruction	623/11.11	428/304.4 ; 442/123; 530/354; 530/356	

	Inventor	S	C	P	2	3	4	5	Image Doc. Displayed	PT
1	Bell; Eugene et al.	X							US 6179872	